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NATIONAL EUTROPHICATION SURVEY METHODS FOR LAKES SAMPLED IN 1972

Pacific Northwest Environmental
Research Laboratory

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16. ABSTRACT

Chemical, physical and biological methods and other procedures used by the U.S. Environmental Protection Agency's National Eutrophication Survey are given. These procedures were used during the first year of the Survey in lake, stream and municipal sewage treatment plant sampling and analysis.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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NATIONAL EUTROPHICATION SURVEY METHODS FOR LAKES SAMPLED IN 1972

WORKING PAPER NO. 1

NATIONAL EUTROPHICATION SURVEY
PNERL, CORVALLIS, OR
NERC, LAS VEGAS, NV
OCTOBER, 1974

TABLE OF CONTENTS

	Page Number
Introduction	1
Participating Laboratories	2
Selection of Lakes	3
Field Sampling Methods	5
Lakes	5
Municipal Sewage Treatment Plants	8
Tributaries and Outlets	9
Analytical Methods	11
Nutrient Analysis	11
USGS Estimates of Stream Flows and Drainage Areas	11
Estimates of Nutrient Loadings	13
Tributaries and Outlets	13
Municipal Sewage Treatment Plants	19
Septic Tanks	20
Precipitation	21
Algal Assay	22
Algal Enumeration and Identification	23
Quality Control	23
PNERL	23
NERC-Las Vegas	24
Interlaboratory	24
Literature Cited	25
Appendix	26

TABLES

	Page Number
TABLE 1 - National Eutrophication Survey Responsibilities.....	2
TABLE 2 - Number of Lakes and Start-Up Dates of Stream Sampling in Northeastern States.....	4
TABLE 3 - Analytical Methods and Precision of Laboratory Analyses.....	12
TABLE 4 - A Summary of Accuracy of Flow Estimates and Drainage Area Measurements Provided by U.S. Geological Survey.....	14

INTRODUCTION

The National Eutrophication Survey (NES) was initiated in 1972 in response to an Administration commitment to investigate the nationwide threat of accelerated eutrophication to fresh water lakes and reservoirs. The Survey is designed to develop, in conjunction with state environmental agencies, information on nutrient sources, concentrations, and impact on selected fresh water lakes as a basis for formulating comprehensive and coordinated national, regional, and state management practices relating to point-source discharge reduction and nonpoint-source pollution abatement in lake watersheds.

Sampling was initiated in ten northeastern states (Minnesota, Michigan, Wisconsin, New York, Connecticut, Massachussetts, Rhode Island, Delaware, New Hampshire, and Maine) in May 1972. This working paper describes the field and laboratory methods which were used in lake, stream, and municipal sewage treatment plant sampling and analysis for these states.*

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*Due to substantial changes in equipment, techniques, and analyses performed in subsequent years of the Survey, this document should be utilized only with data collected from the above ten states.

PARTICIPATING LABORATORIES

Each of the participating laboratories, the Pacific Northwest Environmental Research Laboratory-Corvallis (PNERL), the National Environmental Research Center-Las Vegas (NERC-LV), and the Headquarters Staff, National Eutrophication Survey, Office of Research and Development, Washington, D.C. (NES/HQS), and others have clearly defined roles in the functional organization of the Survey. Table 1 lists the primary responsibilities of the participating agencies, laboratories, and/or individuals.

SELECTION OF LAKES

Lakes and reservoirs which were included in the Survey in 1972 were selected through discussions with state water pollution agency personnel and EPA Regional Offices. The following selection criteria were used:

1. the lake was impacted by a municipal sewage treatment plant (MSTP) effluent either by direct discharge to the lake or by discharge to a tributary within approximately 25 miles of the lake;
2. was 100 surface acres or greater in size;
3. had a mean hydraulic residence time of no less than 30 days.

Specific selection criteria were waived for some lakes of particular state interest.

Table 2 indicates the number of lakes selected in each state, the number of associated stream sites and MSTP effluents sampled, and the date stream sampling was initiated in each state. Stream sampling continued for 12 months following start-up. The Appendix lists, by state and county, those lakes and reservoirs sampled in 1972.

TABLE 1

NATIONAL EUTROPHICATION SURVEY RESPONSIBILITIES

FUNCTION	PNERL	NERC-LV	NES/HQS	OTHERS
I. Planning and Coordination	X	X	X	
II. Selection of Lakes				
A. Preliminary			X	1,2
B. Final	X	X	X	
III. Lake Sampling				
A. Procedures		X		
B. Sample procurement		X		
C. Field analyses		X		
D. Preliminary lake evaluation		X		
E. Aircraft support		X		
F. Sample and data handling		X		
IV. Tributary Sampling				
A. Procedures	X			
B. Sample procurement	X			1,3
C. Sample and data handling	X			
D. Stream flow data	X			4
V. Sewage Treatment Plant Sampling				
A. Procedures	X			
B. Sample procurement	X			5
C. Sample and data handling	X			
VI. Chemical Analyses				
A. Lake samples		X		
B. Tributary samples	X			
C. Sewage treatment plant samples	X			
D. Quality control				
1. Within lab	X	X		
2. Interlab	X			
VII. Biological Analyses				
A. Chlorophyll a		X		
B. Phytoplankton identification		X		6
C. Algal Assay Procedure	X			
D. Pathogenic protozoa, bacteria		X		7,8
VIII. Land-Use Studies				
A. Watershed selection	X			
B. Imagery acquisition	X			
C. Imagery interpretation	X			
IX. Data Analyses and Report Preparation	X	X	X	1,2*

1. State pollution control agency
2. EPA Regional Office
3. State National Guard Units
4. U.S. Geological Survey
5. Municipal sewage treatment plant operators
6. Dr. Charles Goldman, University of California at Davis, contractor
7. Dr. Shih L. Chang, Water Supply Research Laboratory, Cincinnati, Ohio
8. Dr. Victor Cabelli, Northeast Water Supply Laboratory, Narragansett, Rhode Island

*Review and Comment

TABLE 2

NUMBER OF LAKES AND START-UP DATES
OF STREAM SAMPLING IN NORTHEASTERN STATES

STATE	NUMBER OF NES LAKES	NUMBER OF STREAM SITES	NUMBER OF SAMPLED WASTE EFFLUENTS	MONTH AND YEAR STREAM SAMPLING INITIATED*
Vermont	7	52	23	July 1972
Connecticut	8	74	17	August 1972
Rhode Island	3	28	1	August 1972
New Hampshire	4	52	5	August 1972
Massachusetts	8	37	15	September 1972
Maine	9	59	5	September 1972
Wisconsin	46	170	16	September 1972
Minnesota	74	231	56	October 1972
Michigan	37	170	51	October 1972
New York	25	242	36	November 1972

*Lake sampling completed in 1972 by EPA, NERC-LV

FIELD SAMPLING METHODS

Lakes

Lakes and sampling sites were located using U.S. Geological Survey (USGS) 7 1/2' quadrangles whenever possible. When these were not available, 15' or 1:250,000 series USGS maps were employed. In some instances bathymetric maps were obtained through the assistance of state or regional agencies; these were invaluable in selecting sampling sites. Unfortunately, many of the lakes included in the Survey either had not been bathymetrically mapped or the maps could not be obtained prior to sampling.

Sampling sites were selected based upon available information on lake morphometry, potential major sources of nutrient input, and the on-site judgment of the limnologist on the helicopter. Primary sampling sites were chosen to reflect the deepest portion of each major basin in a test lake. Where many basins were present, selection was guided by nutrient source information on hand. The number of sampling sites for a test lake was limited commensurate with the survey nature of the program. After selection, site locations were marked and numbered on a quadrangle map. From these, map coordinates were determined and entered on a site-description form. Occasionally a sampling location was modified, deleted, or added because of subsequent information received relevant to the basic site-selection criteria.

Lake sampling was accomplished by two sampling teams, each consisting of a limnologist, pilot, and sampling technician, operating from pontoon-equipped Bell UH-1H helicopters. A mobile field laboratory provided analytical support.

The helicopters were equipped with electric winches and approximately 200 feet of hollow-core, multi-conductor cable attached to a submersible pump and an Interocean Systems sensor capable of making *in situ* measurements of conductivity, temperature, optical transmissivity, dissolved oxygen, pH, and depth. Unfortunately, the pH and dissolved oxygen sensors were often inoperative necessitating collection of physical water samples for these parameters. An echo sounder, Secchi disc, sample containers and related equipment items necessary for water sampling were also carried in each helicopter.

After landing at the approximate site, the helicopter was water-taxied in the area to locate the deepest nearby water. There a small buoy was deployed to serve as a reference to the pilot for maintaining the helicopter on station. Compass bearings were taken to prominent landmarks from each sample site to permit return to the same location on subsequent sampling rounds. Observations were recorded concerning general lake appearance, phytoplankton bloom conditions, and shoreline development; Secchi disc readings were made, and bucket-dipped surface water samples were collected.

After the sensor-pump package was immersed, sensor outputs were checked and the sensor was lowered slowly through the water column until it contacted bottom. It was then raised to a point four feet off the bottom to avoid pump damage from sediments entering the intake. The digital readout of each sensor at that depth was recorded and the submersible pump was activated. Water samples were collected after allowing sufficient time for the pump to completely purge the hollow cable of water from the previous station. By that time, depths had been selected for the collection of other water samples that would best represent the water column. Upon completion of sampling near

the bottom, the sensor was raised to the next level, digital values were recorded, and water samples pumped. This process was repeated at each depth selected for collection of water samples at a given site.

Integrated samples for algal identification or chlorophyll-a analysis were collected by continuing to pump while raising or lowering the sensor package. Water collection was timed to provide a uniform mixture of water from the surface to 15 feet, or to a point just off the bottom within water less than 15 feet deep.

At each sampling depth water samples were collected for nutrient, alkalinity, pH, conductivity, and dissolved oxygen determinations. For nutrient analysis two eight-ounce sample bottles were filled and the samples immediately preserved with mercuric chloride.

Samples for dissolved oxygen determinations were collected in 300 ml BOD bottles, immediately fixed with Hach powder pillow reagents, and stored out of direct sunlight. Samples for pH, conductivity, and chlorophyll-a analyses were collected in polyethylene bottles and refrigerated in the dark until completion of the day's sampling operation. Algal identification samples were preserved with acid Lugol's solution aboard the helicopter.

Conductivity and pH electrode determinations were made as soon as possible following their return to the field laboratory at the end of the day's sampling. A Beckman Electromate portable pH meter and combination electrode were used to determine pH. Conductivity measurements were made with a Beckman Model RB338 conductivity bridge. These were utilized primarily as a check on the in situ sensors, but, on occasions, were the primary data. Dissolved oxygen samples were titrated with phenylarsine oxide in the mobile field laboratory within 16 hours after collection.

Chlorophyll-a analyses were performed at the end of each day in the trailer laboratory according to the fluorometric procedure described by Yentsch and Menzel (1963).* One of each pair of nutrient samples was filtered through a 0.45 micron HA Millipore filter into a clean polyethylene bottle, recapped, and, along with its unfiltered counterpart, forwarded to NERC-LV for analysis.

During the last sampling period, a five-gallon algal assay sample was obtained by compositing water collected at each sampling level and combining such samples from each site on a lake. If a lake had more than four stations, groups of sites were combined into two or more polyethylene cubitainers. The unpreserved algal assay samples were forwarded to PNERL for processing.

Municipal Sewage Treatment Plant

With the cooperation of state agencies, an attempt was made to identify all municipal sewage treatment plants (MSTP) discharging directly or indirectly into each lake. The operator at each MSTP was requested to provide monthly effluent samples for a period of one year. Each operator who agreed to cooperate was provided with a sampling kit including instructions, sample containers, sample labels, mercuric chloride preservative, shipping boxes, and pre-addressed, franked shipping labels.

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*Chlorophyll-a analyses during 1972 were often delayed due to lack of sufficient personnel. Subsequent studies suggest that short-term frozen storage of filtered samples can give rise to chlorophyll-a losses in excess of 20%: The data listings in STORET must be considered subject to a potential error of this magnitude.

He was asked to provide one of the following samples (listed in order of descending preference):

1. a once-monthly 24-hour composite sample (proportional composite if flows were metered or measured),
2. a once-monthly 8-, 10-, or 12-hour composite sample (proportional if flows were metered or measured),
3. a once-monthly modified composite sample consisting of about 500 ml collected at 11 a.m. and another 500 ml collected at 4 p.m. of the same day, or
4. a once-monthly single grab sample of about one liter collected on a weekday between the hours of 8 A.M. and 8 P.M.

Following collection, the plant operator preserved the sample by adding to it the contents of a vial containing sufficient mercuric chloride to achieve a concentration of 400 mg/l in the sample. The sample label was completed by the sample collector and included data on sample type, date, mean flow for the day of sampling, and mean daily flow for the month in which the samples were collected. Samples were mailed to PNERL for analysis.

Tributaries and Outlets

Sampling sites were selected on significant tributaries to each lake near the point where the tributary discharged to the lake. Where municipal waste discharges were located on tributaries, sampling sites were also designated upstream from the point of effluent discharge. Sampling sites for outlets of lakes or reservoirs were located at the nearest feasible sampling point downstream from the water body being surveyed.

Monthly samples at the designated stream sites were collected through the volunteer efforts of the National Guard in each of the involved states. When sampling was started in any of the states, a scientist from EPA or the state agency accompanied each National Guard sampling team to each site during the first sampling to train the team in proper techniques of sample collection, preservation and handling. During the first sampling, the unique six-digit station number was stenciled on the bridge or another permanent landmark at the sampling site to insure positive identification of the site. Subsequent monthly sampling for a period of one year, plus two additional samples during high flow periods, was done exclusively by the National Guard sampling teams.

Stream samples were collected in clean, previously unused, wide-mouth, one-liter polyvinyl chloride bottles. These were inserted in a sampler consisting of a section of plastic pipe with a cross-bar bottle retainer at one end and rubber tubing stretched across the other end to secure the bottle. A rope was attached to the sampler to lower it from a bridge or stream bank to the water surface. The water collected in the sample bottle at each site was essentially a surface grab sample, although the sampling rig was lowered to mid-depth in the stream before it was retrieved.

Following sample collection at each site, the Guard team completed a label attached to each bottle recording the stream name, station number, date, time, and signature of the individual responsible for collecting the sample. The sample was preserved at the site with mercuric chloride. Following inventory at the Guard base, samples were sent to PNERL for analysis.

ANALYTICAL METHODS

Nutrient Analyses

The analytical methods utilized to process the samples at both NERC-Corvallis and NERC-LV are presented in Table 3. All of the analyses were performed utilizing adaptations of automated procedures described in "Methods for Chemical Analysis of Water and Wastes" (EPA, 1971).

It should be noted that there were some differences in the analyses performed at each laboratory. The lake water samples were analyzed at NERC-LV for total phosphorus, total dissolved phosphorus, nitrite-nitrate-N, ammonia-N, and total alkalinity. The stream and waste water effluent samples were processed at NERC-Corvallis where total phosphorus, dissolved orthophosphorus, nitrite-N, nitrate-N, nitrite-nitrate-N, ammonia-N, and total Kjeldahl nitrogen analyses were performed.

USGS ESTIMATES OF STREAM FLOWS AND DRAINAGE AREAS

For each sampled stream, the various District Offices of the USGS made estimates of the mean flow for the day of sampling, the flow for each month of sampling, and the "normalized" mean flow for each month (i.e., flows expected during a period of average precipitation and hydrology). In addition, runoff estimates were made for the unsampled portion of the total watershed of each lake and the area of the drainage basin for each sampled tributary stream and for each lake or reservoir was provided.

TABLE 3

ANALYTICAL METHODS AND
PRECISION OF LABORATORY ANALYSES*

PARAMETER		
Dissolved Orthophosphate	Single reagent methods involving colorometric determination of antimony-phosphomolybdate complex.	±0.005 mg/l or ±5%
Total Dissolved Phosphorus	Filtration and persulfate oxidation followed by the above method for dissolved orthophosphate.	±0.005 mg/l or ±5%
Total Phosphorus	Persulfate oxidation followed by the above method for dissolved orthophosphate.	±0.005 mg/l or ±5%
Nitrite-N	Diazotization of sulfanilamide by nitrite coupled with N-(1-naphthyl)-ethylene diamine.	±0.001 mg/l or ±2%
Nitrite-Nitrate-N	Cadmium reduction followed by the above method for Nitrite-N.	±0.010 mg/l or ±5%
Nitrate-N	Determined by difference between the preceding two reactions.	±0.010 mg/l or ±5%
Ammonia-N	Alkaline phenol hypochlorite reaction producing indophenol blue.	±0.005 mg/l or ±5%
Kjeldahl-N	Acid digestion followed by the above procedure for ammonia nitrogen.	±0.10 mg/l or ±5%
Total Alkalinity	Methyl orange colorometric.	±0.5 or ±5%

*Although the % precision value does not change, wastewater analyses precision values are an order of magnitude higher than those expressed (i.e. ±0.05 mg/l vice ±0.005 mg/l).

In some instances, flow gages were present at sampling sites or within a reasonable distance and were used to provide flow estimates. In cases where sampled tributaries were ungaged, flow estimates were based on runoff patterns at the nearest gaged stream system. Where available, flow information was also obtained from other sources, such as the Corps of Engineers or power companies which maintain records of reservoir discharge.

The errors in drainage area measurements and flow estimates varied from one area to another and were highly dependent on the availability of topographic maps of the appropriate scale, the number of gaged stream sites for a given lake system, land relief, and other factors. In general, measurements or estimates which were provided by USGS for the larger drainage areas were the best, since these were subject to less severe fluctuations in stream flow within a given period of time.

Table 4 summarizes the accuracy of flow estimates and drainage area calculations by state as provided by the District Offices of the USGS.

ESTIMATES OF NUTRIENT LOADINGS

Tributaries and Outlets

Lake tributary and outlet nutrient loads included in each of the lake reports were estimated for a "normalized" or average flow year rather than for the year in which samples were collected. This approach was used because it was deemed more important to determine what sewage treatment plant contributions or land-runoff contributions were under average conditions rather than during any extreme hydrological conditions which may have occurred during the year of sampling.

TABLE 4

A SUMMARY OF ACCURACY OF FLOW ESTIMATES AND DRAINAGE AREA MEASUREMENTS PROVIDED BY U.S. GEOLOGICAL SURVEY

State	Drainage Areas	Mean Daily Flow	Mean Monthly Flow	Normalized Mean Monthly Flow	Mean Annual Flow
Connecticut	±1%	gaged ±10% ungaged ±20%	gaged ±10% ungaged ±20%	gaged ±10% ungaged ±12% high flow ungaged ±27% low flow	
Maine	±1%	gaged ±5% ungaged ±200%	gaged ±5% ungaged ±30% (>20 mi^2) ±75% (<20 mi^2)	gaged ±5% ungaged ±20% (>20 mi^2) ±50% (<20 mi^2)	gaged ±5%
	Maine ungaged accuracies are for worst low flow conditions and would be substantially better for much of the year.				
Massachusetts	±1%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	
Michigan	±5%	Estimates provided for 74% of the sites had accuracy of ±25%. Remainder of sites up to ±40%.	Slightly better accuracy the daily estimates.	Slightly better accuracy than daily estimates.	Slightly better accuracy than daily estimates.
Minnesota	±5% with a few ±10%	gaged ±10% ungaged ±25-50% with greater error for D.A. <10 mi^2	gaged ±5% ungaged ±10-25% with 50% for D.A. <10 mi^2	gaged ±5% ungaged - same as mean monthly	gaged ±5% ungaged - same as mean monthly
New Hampshire	±1%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%
New York	±5%, except for small basins at ±10%	Estimates provided for 79% of the sites had accuracy of ±5-25%. Remainder of sites up to ±50%.	Better than daily.	±15%	No value

(TABLE 4 - Continued)

State	Drainage Areas	Mean Daily Flow	Mean Monthly Flow	Normalized Mean Monthly Flow	Mean Annual Flow
Rhode Island	±1%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%
Vermont	±1%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	gaged ±15% ungaged ±20%	gaged ±15%
Wisconsin	±0.5%	±40%	Somewhat better than daily flows	Average ±35%	No value

Normally, 14 samples were collected from each stream site. Occasionally the number of data points was less than 14 due to a sample or two not being collected during winter ice conditions, sample loss, breakage, or laboratory error. Although these are adequate data to provide a reasonable estimate of the average concentration for a given stream for the sampled year, the data from any one site are not adequate to satisfactorily estimate the relationship between flow and concentration at that site. Variations in flow both within and between years make it unsatisfactory to obtain a loading estimate simply by multiplying the observed average concentration times the annual normalized flow.

The procedure used was to estimate from combined data on a large number of streams the extent of the relationship between concentration and flow for each nutrient. The value so estimated represented the percent change in concentration resulting from a given percent change in flow. This relation does seem to be reasonably constant from stream to stream, although different for the two major nutrients (a stronger relation for phosphorus than for nitrogen). The appropriate statistical procedure for estimating this parameter is to compute the average slope from a large number of linear regressions, for individual streams, of log concentration on log flow. This was carried out using 250 sampling sites in northeastern and northcentral states. It was found that, on the average, a 1% change in flow results in a -0.11% change in phosphorus concentration and a -0.06% change in nitrogen concentration.

The method of estimating loading was essentially to use these estimated relationships to adjust the concentrations to what they would have been for each month under normalized flow conditions, and then add up the estimated loadings for the 12 months. The annual nutrient load, expressed as

pounds/year, was thus calculated by:

$$\text{Annual load} = 164.502\ \bar{c}\ Y\ S \sum_{1}^{12} NF_i$$

Where:

164.502 = factor including average number of days per month and conversion of concentration and flow to pounds per day,

$\bar{c}$ = mean nutrient concentration in the sampled stream,

NF_i = normalized flow for i^{th} month,

$$Y = 10^{b\{\overline{\log NF} - \overline{\log MF}\}},$$

$$S = \{\sum_{1}^{12} NF_i \cdot 10^{b(\log NF_i - \overline{\log NF})}\} / \sum_{1}^{12} NF_i,$$

$\overline{\log NF}$ = mean log normalized flow,

$\overline{\log MF}$ = mean log monthly flow for year sampled,

and b = -0.11 for phosphorus, -0.06 for nitrogen.

The "Y" factor adjusts the data to account for the fact that the year in which the samples were collected may have been extremely wet or dry which would have had an influence on measured contributions. The "S" factor adjusts the data to account for seasonal flow variations.

The net result of the regression analysis and the formula is an annual loading value which is generally within a few percent of the loading which would be estimated if it were assumed that nutrient concentrations did not vary with changes in stream flow.

In analyzing the data for a tributary having a point source upstream from the sampling point, the total stream load was estimated first by the method detailed above. If the point source was located reasonably close to the sampling site, the total annual contribution to the stream was subtracted from the total nutrient load at the sampling site, and the difference was attributed to nonpoint-source input. If the point source was located several miles upstream from the sampling point, the scientist determining the nutrient loadings analyzed the total stream load (including the point source), the magnitude of the point-source load, and the nonpoint-source load of other stream systems in the area to determine what portion of the nutrient load at the sampling site could logically be attributed to the point source and subtracted from the total stream load. This procedure was not standardized and was performed on an individual basis for each stream system. However, the general rule was to assume that 100% of the point-source load eventually reached the lake or reservoir.

Sampled streams usually included most, but not all, of the lake watershed. Unsampled streams, if any, and drainage from the lakeshore area also contributed nutrients. The nutrient contribution of the unsampled portion of the drainage area was estimated by using the average nutrient export per unit area of sampled stream drainage and multiplying that by the area of the unsampled portion.

Some judgment factors were included in this estimate and how it was made. If point sources strongly influenced one or more sampled streams in a particular lake system, the scientist may have selected nutrient export values from a single stream to estimate loadings from the unsampled portion of the drainage area.

Variations from the above procedure, if any, are noted in the individual lake reports.

Municipal Sewage Treatment Plants

If the operator of a MSTP impacting a surveyed lake submitted effluent samples for analysis, the results were used to estimate nutrient discharge. For these sampled plants, the nutrient loads per million gallons of effluent were calculated for each day of sampling and averaged for the total sampling period. Mean daily flows for each month of sampling were also averaged and the total annual loads in lbs/year were estimated according to the following equation:

$$\text{Annual Load} = (D)(F)(365)$$

where: D = Mean daily load in pounds per million gallons.
F = Mean daily flow in million gallons.

If a plant was not sampled, the nutrient loads were estimated on the basis of sewered population or the 1970 census figures for the municipality if no better sewered population estimate could be obtained. Flows were estimated at 100 gallons/capita/day.

For areas not under a phosphate detergent ban, the following per capita estimates of total phosphorus and nitrogen contributions were used:

	Total P (lbs/capita/yr)	Total N (lbs/capita/yr)
Treated Waste	2.5	7.5
Raw Waste	3.5	9.4

The 3.5 lbs total P/capita/year for raw waste discharge was taken from Bartsch (1972). For treated waste it was assumed that regardless of treatment type, approximately 29% of the total phosphorus would be removed leaving a contribution of 2.5 lbs/capita/year.

The nitrogen value of 7.5 lbs/capita/year was derived from the information that nitrogen to phosphorus ratios in wastewater range from 3-6 (Bartsch, 1972) and that, on the average, treatment removes only 20% of the total nitrogen.

Septic Tanks

Whenever data on the number of lakeshore septic tanks or septic tank nutrient contributions were available, which was infrequently, they were used. In the absence of any given data, the number of dwellings within 100 yards of the lake were counted on the most recent USGS quadrangle map. It was assumed that on a year-long average, 2.5 people occupied each dwelling. Where lakeshore resorts, parks, and/or campgrounds were known, it was assumed that all were served by septic tanks and that each resort was the equivalent of ten dwellings, that the population of each park was 25 persons per day for four months, and that the population of each camp was 50 persons per day for four months.

It was also assumed that after septic tank treatment and discharge to the adsorption field that only 0.25 lbs P/capita/year reached the lake. For nitrogen, which is less amenable to removal by treatment or by adsorption to soil particles, it was assumed that 100% of the nitrogen or 9.4 lbs N/capita/year reached the lake from septic tank systems on the lakeshore.

Precipitation

A figure of 9.634 lbs of total nitrogen/acre lake surface/year was used as an estimate of nitrogen in precipitation. The estimate was the average result reported by Weible (1969) and Corey, et al. (1967) for areas receiving approximately 30 inches of rainfall per year.

An estimate of 0.156 lbs total phosphorus/acre/year was used to represent total phosphorus in precipitation. This estimate, which is probably conservative, lies between the number reported by Corey, et al. (1967) for soluble phosphorus and the lower end of the range reported by Weible (1969) for the Cincinnati, Ohio area.

ALGAL ASSAY

Upon arrival at the laboratory, the algal assay samples were frozen until processing could begin. The procedures used in the algal assay test were basically those outlined in the publication entitled Algal Assay Procedure Bottle Test (EPA, 1971). Each sample was autoclaved at 121°C and 15 psi for 10-30 minutes to kill indigenous organisms and solubilize nutrients bound by particulate matter and was then filtered. Chemical analyses for nutrients and other constituents were performed before and after autoclaving.*

Each lake water sample was treated with several nutrient concentrations in separate flasks. In addition, a lake water control with no nutrient supplement was assayed. Nutrient spikes included 0.005 mg/l phosphorus, 0.02 mg/l phosphorus, 0.05 mg/l phosphorus, 10.0 mg/l nitrogen, and a combination of 0.05 mg/l phosphorus plus 10.0 mg/l nitrogen.

After the various nutrient additions had been made to each set of lake water samples, each flask was inoculated with 1,000 cells/ml of the test alga, Selenastrum capricornutum. Following inoculation the cultures were incubated for 14 days at 24°C on gyrorotary shakers under 400 foot-candles of continuous light. Algal growth was monitored throughout the incubation

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*Results of nutrient analyses performed on unpreserved water samples prior to autoclaving often differed substantially from those of corresponding mercuric chloride preserved water samples. Some of these discrepancies may be attributed to the differences in sampling procedures for the two types of samples. Most, however, were due to adsorption onto the container walls during prolonged storage. In particular, significant losses of phosphorus and inorganic nitrogen were often noted. Because of these losses, the algal assay results are somewhat suspect but are believed to be usable if considered in context with inorganic nitrogen: dissolved phosphorus ratios computed from preserved water samples obtained on the date of algal assay sampling.

period by cell counts and mean cell-volume measurements made with Coulter electronic particle counters. The maximum growth attained was quantified in terms of mg/l dry weight equivalents of the cell counts and mean cell volumes.

ALGAL ENUMERATION AND IDENTIFICATION

Identification and differential counts of phytoplankton genera were made with a microscope and counting chamber. The differential counts, converted to organisms per ml, were entered into a WYLBUR computer file to increase accessibility and facilitate use of the data in trophic indices. The five most prevalent genera and total counts per ml were tabulated for each lake sampled. Type slides and photomicrographs were prepared to document specific and unique phytoplankton assemblages.

QUALITY CONTROL

?NERL

Quality control began with the receipt of the sample. After all identifying information was logged, a laboratory number was assigned identifying the sample and the analyses to be performed.

The data entered on laboratory request forms were teletyped to the Oregon State University computer and processed through the sample handling and verification system (SHAVES) program (Krawczyk and Byram, 1973).

At the request of the analyst, the SHAVES program produces a "run list" for samples indicating, by laboratory number, the sequence in which the sample

should be analyzed and also which samples should be replicated and/or spiked with known quantities of the material being analyzed. The run list usually specified a set of standards, 120 samples, and then another set of standards. Every eighth sample was replicated, and every twentieth sample was spiked.

Analytical data readouts were entered into the computer which performed a check on calculations and analytical accuracy and precision.

Blind samples (ten sub-samples identified as separate samples) were sent through the system to check both the analysts and the equipment. Scheduled replicate samples provided regular checks on analytical procedures.

NERC-Las Vegas Laboratory

In the Laboratory Operations Branch at NERC-Las Vegas every twentieth sample was replicated and also spiked with a known amount of the constituent being analyzed. An average of 15 blind samples per month were sent through the laboratory as a check on analysts and instrumentation. Samples of known of the analytical process.

Interlaboratory

Samples for interlaboratory comparison originated from several sources including NES lake or stream samples, Methods Development and Quality Assurance Research Laboratory, NERC-Cincinnati (MDQARL) reference samples and unknown material furnished by International Field Year - Great Lakes personnel. The results of the 1973 interlaboratory testing program for various forms of nitrogen and phosphorus showed no significant differences between laboratories, and compare very favorably with interlaboratory comparisons for nutrients presented in Method Study 2 of the MDQARL.

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APPENDIX

LAKE LISTS

APPENDIX

CONNECTICUT

STORET #	LAKE	COUNTY
0901XX	Aspinook Pond	New London, Windham
0902XX	Bantam Lake	Litchfield
0903XX	Community Lake	New Haven
0904XX	Eagleville Lake	Tolland
0905XX	Hanover Pond	New Haven
0910XX	Zoar Lake	Fairfield, New Haven
0911XX	Lillinoah Lake	Litchfield, Fairfield, New Haven
0912XX	Shelton Lake	Fairfield

MAINE

STORET #	LAKE	COUNTY
2304XX	Estes Lake	York
2306XX	Long Lake (Bridgeton)	Cumberland
2308XX	Mattawamkeag Lake	Aroostock
2309XX	Moosehead Lake	Piscataquis, Somerset
2310XX	Rangeley Lake	Franklin, Oxford
2311XX	Sebago Lake	Cumberland
2312XX	Sebasticook Lake	Penobscot
2313XX	Long Lake (St. Agatha)	Aroostock
2314XX	Bay of Naples	Cumberland

MASSACHUSETTS

STORET #	LAKE	COUNTY
2502XX	Hager Pond	Middlesex
2503XX	Harris Pond	Worcester (Portion in Providence Co., R.I.)
2504XX	Maynard Impoundment	Middlesex
2507XX	Woods Pond	Berkshire
2508XX	Mattfield Impoundment	Plymouth
2509XX	Rochdale Pond	Worcester
2510XX	Grist Millpond	Middlesex
2511XX	Billerica Impoundment	Middlesex
2512XX	Northboro Impoundment	Worcester
2513XX	Hudson Impoundment	Middlesex

NEW HAMPSHIRE

STORET #	LAKE	COUNTY
3302XX	Powder Mill Pond	Hillsborough
3303XX	Lake Winnipesaukee	Carroll, Belknap
3305XX	Kelly Falls Pond	Hillsborough
3306XX	Glenn Lake	Hillsborough

NEW YORK

STORET #	LAKE	COUNTY
3602XX	Black Lake	St. Lawrence
3603XX	Canadarago Lake	Otsego
3604XX	Canandaigua Lake	Ontario, Yates
3606XX	Carry Falls Reservoir	St. Lawrence
3607XX	Cassadaga Lake	Chautauqua
3608XX	Cayuga Lake	Tompkins, Cayuga, Seneca
3609XX	Champlain Lake	Essex, Clinton, Washington
3610XX	Chautauqua Lake	Chautauqua
3611XX	Cross Lake	Onondaga, Cayuga
3613XX	Goodyear Lake	Otsego
3615XX	Huntington Lake	Sullivan
3616XX	Irondequoit Bay	Monroe
3617XX	Keuka Lake	Steuben, Yates
3619XX	Long Lake	Hamilton
3622XX	Oneida Lake	Madison, Oneida, Oswego, Onondaga
3623XX	Onondaga Lake	Onondaga
3625XX	Otter Lake	Cayuga
3627XX	Owasco Lake	Cayuga
3629XX	Raquette Pond	Franklin, St. Lawrence
3630XX	Round Lake	Saratoga

New York - Continued

STORET #	LAKE	COUNTY
3631XX	Rondout Reservoir	Sullivan, Ulster
3632XX	Sacandaga Reservoir	Fulton, Saratoga, Hamilton
3633XX	Saratoga Lake	Saratoga
3634XX	Schroon Lake	Essex, Warren
3635XX	Seneca Lake	Schuyler, Ontario, Seneca, Yates
3636XX	Swan Lake	Sullivan
3637XX	Swinging Bridge Reservoir	Sullivan
3639XX	Conesus	Livingston
3640XX	Lower St. Regis	Franklin
3641XX	Allegheny	Cattaraugus (Portion in Pa.)

RHODE ISLAND

STORET #	LAKE	COUNTY
4402XX	Slatersville Reservoir	Providence
4403XX	Turner Reservoir	Providence (Portion in Bristol Co., Mass.)

VERMONT

STORET #	LAKE	COUNTY
5001XX	Champlain Lake	Rutland, Addison, Chittenden, Franklin, Grand Isle (See New York listings)
5002XX	Clyde Pond	Orleans
5005XX	Harriman Reservoir	Windham
5007XX	Lamoille Lake	Lamoille
5008XX	Memphremagog Lake	Orleans (Portion in Canada)
5010XX	Arrowhead Mountain Lake	Chittenden, Franklin
5011XX	Waterbury Reservoir	Washington, Lamoille

MICHIGAN

STORET #	LAKE	COUNTY
2603XX	Lake Allegan	Allegan
2606XX	Barton Lake	Kalamazoo
2609XX	Belleville Lake	Wayne
2610XX	Betsie Lake	Benzie
2613XX	Brighton Lake	Livingston
2617XX	Lake Charlevoix	Charlevoix
2618XX	Lake Chemung	Livingston
2621XX	Lake Constantine	St. Joseph
2624XX	Deer Lake	Marquette
2627XX	Fallassburg Reservoir	Ionia
2629XX	Ford Lake	Washtenaw
2631XX	Fremont Lake	Newaygo
2640XX	Jordan Lake	Ionia, Barry
2643XX	Kent Lake	Oakland, Livingston
2648XX	Lake Macatawa	Ottawa
2649XX	Manistee Lake	Manistee
2659XX	Muskegon Lake	Muskegon
2665XX	Pentwater Lake	Oceana
2669XX	Portage Lake	Houghton
2671XX	Randall Lake	Branch
2672XX	Rogers Pond	Mecosta
2673XX	Ross Lake	Gladwin
2674XX	Sanford Lake	Midland
2679XX	Sturgeon Lake	Dickinson

Michigan - Continued

STORET #	LAKE	COUNTY
2683XX	Thornapple Lake	Barry
2685XX	Union Lake	Branch
2686XX	Victoria Dam	Ontonagon
2688XX	White Lake	Muskegon
2691XX	Mona Lake	Muskegon
2692XX	Long Lake	St. Joseph
2693XX	St. Louis Reservoir	Gratiot
2694XX	Crystal Lake	Montcalm
2695XX	Higgins Lake	Roscommon, Crawford
2696XX	Houghton Lake	Roscommon
2697XX	Thompson Lake	Livingston
2698XX	Pere Marquette Lake	Mason
2699XX	Strawberry Lake	Livingston
26A0XX	Holloway Reservoir	Lapeer, Genesse
26A1XX	Caro Reservoir	Tuscola
26A2XX	Boardman Hydro Pond	Grand Traverse

MINNESOTA

STORET #	LAKE	COUNTY
2701XX	Addie Lake	McLeod
2702XX	Albert Lea Lake	Freeborn
2704XX	Badger Lake	Polk
2705XX	Bartlett Lake	Koochiching
2706XX	Bear Lake	Freeborn
2708XX	Big Lake	Stearns
2709XX	Big Stone Lake	Big Stone (Portion in South Dakota)
2710XX	Birch Lake	Cass
2711XX	Blackduck Lake	Beltrami
2712XX	Blackhoof Lake	Crow Wing
2713XX	Buffalo Lake	Wright
2714XX	Carrigan Lake	Wright
2715XX	Cass Lake	Beltrami, Cass
2716XX	Clearwater Lake	Wright, Stearns
2717XX	Clitherall Lake	Otter Tail
2719XX	Cokato Lake	Wright
2720XX	Cranberry Lake	Aitkin
2722XX	Deer Lake	Anoka
2725XX	Elbow Lake	St. Louis
2727XX	Elk Lake	Sherburne
2728XX	Embarrass Lake	St. Louis
2730XX	Fall Lake	St. Louis

Minnesota - Continued

STORET #	LAKE	COUNTY
2731XX	Fanny Lake	Douglas
2737XX	Gull Lake	Cass
2739XX	Heron Lake	Jackson
2745XX	Lake of the Woods	Roseau, Lake of the Woods (Portion in Canada)
2746XX	Leech Lake	Cass
2747XX	Lily Lake	Blue Earth
2748XX	Little Lake	Grant
2749XX	Whitewater Lake	St. Louis
2750XX	Madison Lake	Blue Earth
2751XX	Menomin (Mahnomen)	Crow Wing
2752XX	Malmedal Lake	Pope
2753XX	Maple Lake	Douglas
2756XX	Mashkenode Lake	St. Louis
2757XX	McQuade Lake	St. Louis
2758XX	Meuwissen Lake	Carver
2760XX	Minnetonka Lake	Hennepin, Carver
2761XX	Minnewaska Lake	Pope
2765XX	Pelican Lake	St. Louis
2769XX	Portage Lake	Otter Tail
2770XX	Pullman Lake	Grant
2771XX	Rabbit Lake	Crow Wing
2776XX	St Louis Bay	St. Louis
2777XX	Sakatah Lake	Le Sueur, Rice
2780XX	Shagawa Lake	St. Louis

Minnesota - Continued

STORET #	LAKE	COUNTY
2782XX	Silver Lake	McLeod
2783XX	Six Mile Lake	St. Louis
2784XX	South Lake	McLeod
2785XX	St. Clair Lake	Becker
2786XX	Superior Bay	St. Louis
2788XX	Swan Lake	Itasca
2792XX	Trace Lake	Todd
2793XX	Trout Lake	Itasca
2796XX	Tuttle Lake	Martin
2799XX	Willow Lake	Redwood
27A1XX	Winona Lake	Douglas
27A2XX	Wolf Lake	Beltrami, Hubbard
27A3XX	Woodcock Lake	Kandiyohi
27A4XX	Lake Pepin	Goodhue, Wabasha (See Wisconsin listing)
27A5XX	Zumbro Lake	Olmsted, Wabasha
27A6XX	Spring Lake	Washington, Dakota
27A7XX	St. Croix	Washington (See Wisconsin listing)
27A8XX	Budd Lake	Martin
27A9XX	Forest Lake	Washington
27B0XX	White Bear Lake	Ramsey, Washington
27B1XX	Wagongo Lake	Kandiyohi
27B2XX	Green Lake	Kandiyohi
27B3XX	Nest Lake	Kandiyohi

Minnesota - Continued

STORET #	LAKE	COUNTY
27B4XX	Darling	Douglas
27B5XX	Lake Le Homme Dieu	Douglas
27B6XX	Calhoun	Hennepin
27B7XX	Wallmark (Mud) Lake	Chisago
27B8XX	Lost Lake	St. Louis
27B9XX	Lake Carlos	Douglas
27C0XX	Andrusia Lake	Beltrami
27C1XX	Lake Bemidji	Beltrami
27C2XX	Mud Lake (Hasca City)	Itasca
27C3XX	Cottonwood Lake	Lyon
27C4XX	Mud Lake (Wright City)	Wright

WISCONSIN

STORET #	LAKE	COUNTY
5502XX	Altoona Lake	Eau Claire
5503XX	Beaver Dam Lake	Dodge
5508XX	Butte Des Morts Lake	Winnebago
5509XX	Butternut Lake	Ashland, Price
5510XX	Castle Rock Flowage	Juneau, Adams
5513XX	Delavan Lake	Walworth
5515XX	Eau Claire Lake	Eau Claire
5519XX	Green Lake	Green Lake
5520XX	Kegonsa Lake	Dane
5522XX	Koshkonong Lake	Dane, Jefferson, Rock
5531XX	Nagawicka Lake	Waukesha
5532XX	Oconomowoc Lake	Waukesha
5534XX	Petenwell Flowage	Wood, Adams, Juneau
5535XX	Pigeon Lake	Waupaca
5536XX	Pine Lake	Waukesha
5538XX	Poygan Lake	Waushara, Winnebago
5539XX	Shawano Lake	Shawano
5541XX	Sinissippi Lake	Dodge
5545XX	Swan Lake	Columbia
5546XX	Tainter Lake	Dunn
5548XX	Town Line Lake	Oneida
5550XX	Wapogasset Lake	Polk

Wisconsin - Continued

STORET #	LAKE	COUNTY
5551XX	Wausaw Lake	Marathon
5554XX	Winnebago Lake	Fond Du Lac, Calumet, Winnebago
5555XX	Wisconsin Lake	Columbia
5556XX	Wissota Lake	Chippewa
5557XX	Pewaukee Lake	Waukesha
5558XX	Okauchee Lake	Waukesha
5559XX	Tichigan Lake	Racine
5560XX	Browns Lake	Racine
5561XX	Lake Geneva	Walworth
5562XX	Como Lake	Walworth
5563XX	Lac La Belle	Waukesha
5564XX	Rock Lake	Jefferson
5565XX	Big Eau Pleine Reservoir	Marathon, Portage
5566XX	Round Lake	Waupaca
5568XX	Rome Pond	Jefferson
5569XX	Middle Lake	Walworth
5570XX	Grand Lake	Green Lake
5571XX	Crystal Lake	Vilas
5572XX	Trout Lake	Vilas
5573XX	Long Lake	Price
5574XX	Willow Reservoir	Oneida
5575XX	Elk Lake	Price
5576XX	Yellow Lake	Burnett
5577XX	Beaver Dam Lake	Barron

Wisconsin - Continued

STORET #	LAKE	COUNTY
5578XX	St. Croix Lake	St. Croix, Pierce (See Minnesota listing)
5580XX	St. Louis Bay	Douglas (See Minnesota listing)
5581XX	Superior Bay	Douglas (See Minnesota listing)
5582XX	Pepin Lake	Pierce, Pepin, Buffalo (See Minnesota listing)